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TRANSFER OF INSULIN-LIKE GROWTH FACTORS I AND II FROM PLASMA TO LYMPH IN YOUNG GOATS

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SUMMARY

The plasma clearance of intravenously injected ^{125}I-labelled insulin-like growth factor I (IGF-I, $n = 13$) and IGF-II ($n = 12$) and their transfer into lymph draining the foreleg of 3·5- to 8-week-old British Saanen goats was studied. Both peptides were initially distributed into a volume equivalent to the plasma volume, while the final distribution spaces for IGF-I and IGF-II were $90 \pm 9{\cdot}8$ and 125 ± 12 ml/kg live weight respectively. There were two phases to the plasma clearance of both IGF-I and IGF-II, with the half-lives of both phases for IGF-I ($9{\cdot}6 \pm 0{\cdot}9$ and 287 ± 23 min, first and second phase respectively) being significantly ($P < 0{\cdot}001$) longer than those of IGF-II ($4{\cdot}2 \pm 0{\cdot}6$ and 172 ± 18 min, respectively). The radioactivity transferred into lymph originated from intact IGF-I and IGF-II as well as degraded products of these compounds, as assessed by precipitation with trichloroacetic acid and gel filtration. Levels of undegraded IGF-I in lymph were 50% greater than IGF-II. While more than 90% of either peptide was bound to specific IGF-binding proteins in plasma, in lymph 34 ± 2% of IGF-I and 23 ± 3% of IGF-II remained in the free form 60–80 min after injection. The plasma:lymph ratio for free IGF-I was 2:1 and for bound IGF-I, 8:1. The corresponding values for IGF-II were 3:2 and 8:1 respectively. These results provide direct experimental evidence for transfer of undegraded IGF-I and IGF-II from blood into lymph of the foreleg, consistent with the ability of these factors to act in an endocrine role in growing tissues. Differences between plasma clearance and transfer of IGF-II into lymph compared with IGF-I may be due to its greater cellular uptake and/or degradation *in vivo*.

INTRODUCTION

The somatomedin hypothesis proposed by Salmon & Daughaday (1957) stated that the growth-promoting activity of growth hormone is mediated by serum sulphation factors. These were later termed somatomedins (Daughaday, Hall, Raben, Salmon, Van Den Brande & Van Wyk, 1972) and then insulin-like growth factors I and II (IGF-I and IGF-II; Daughaday, Hall, Salmon, Van Den Brande & Van Wyk, 1987). The search for the source of IGFs revealed the majority of tissues have the capacity to produce IGF-I and IGF-II (D'Ercole, Stiles & Underwood, 1984; Matthews, Norstedt & Palmiter, 1986; Murphy, Bell & Friesen, 1987), but blood appears to be the major store of these factors (D'Ercole *et al.* 1984).

IGFs in plasma are bound to specific high-affinity binding proteins which may be separated by gel filtration at positions corresponding to approximate molecular weights of 150 and 40 kDa (see Ooi & Herington, 1988; Baxter & Martin, 1989*a*; Holly & Wass, 1989; Clemmons, 1991). The 150 kDa protein binds most of the circulating IGFs and consists of three subunits; IGF, an acid-stable IGF binding unit and an acid-labile unit which helps

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form the 150 kDa complex but does not in itself bind IGF (Baxter & Martin, 1989*b*). Plasma also contains several binding proteins of approximately 40 kDa which together bind only a small proportion of total circulating IGF. Extravascular fluids, such as lymph or cerebral spinal fluid also contain several binding proteins of around 40 kDa and small amounts of the 150 kDa complex (Ooi & Herington, 1988; Baxter & Martin, 1989*a*; Hodgkinson, Moore, Napier, Davis, Bass & Gluckman, 1989*b*; Lord, Martin, Walton, Ballard & Read, 1991). Accurate measurement of the quantity of unbound IGF in circulation is difficult because of the very small amounts present, but it is thought that this might comprise less than 1% of total circulating IGF (Daughaday, Ward, Goldberg, Trivedi & Kapadia, 1982).

For circulating IGFs to act *in vivo* they must be able to traverse the capillary endothelial barrier between them and the target tissues. Experiments *in vitro* suggest exogenously added IGF-I and IGF-II are internalized by bovine capillary endothelial cells and subsequently released into the medium in an undegraded form (Banskota, Carpentier & King, 1986; Bar, Boes & Yorek, 1986), but whether such a vectorial pathway exists *in vivo* is not known. In the present study we studied the transfer of IGF-I and IGF-II from blood into lymph draining the foreleg of 3- to 8-week-old goats to examine the ability of these peptides to pass undegraded into the extracelluar fluid space of a group of tissues undergoing rapid growth, presumably partly under control of IGFs. Some of these results have been presented elsewhere in a preliminary form (Baucells, Fleet & Prosser, 1989).

METHODS

Materials

Recombinant human IGF-I and IGF-II were obtained from Bachem UK (Saffron Walden, Cambs). TSK Fractogel HW-55(S) and Triton X-100 were purchased from BDH Chemicals Ltd (Poole, Dorset). Sephacryl S-200, Sephadex G-75(F) and Sephadex G-50(F) were supplied by Pharmacia (Uppsala, Sweden). Iodogen was from Pierce Europe B.V. (Oud-Beijerland, Netherlands) and bovine serum albumin (BSA) and tris(hydroxymethyl)aminomethane (Tris) were from Sigma (St Louis, MO, USA). All other chemicals were of reagent grade.

Iodination of IGF-I and IGF-II

IGF-I and IGF-II were iodinated utilizing iodogen similar to the method outlined by Salacinski, McLean, Sykes, Clement-Jones & Lowry (1981). Iodogen was dissolved in dichloromethane to give a final concentration of 1 mg/ml. Aliquots containing 2 μg were added to polypropylene Eppendorf tubes and dried under a stream of nitrogen. IGF-I (2 μg in 40 μl of 10 mM-HCl), 6 μl of a solution of 0·1 M-Tris with 0·5% (vol/vol) Triton X-100 and 5 μl of Na[^{125}I] (0·5 mCi, Amersham International plc, Bucks) was added to the tube of iodogen. The iodination was allowed to continue for 10 min at room temperature, then stopped by adding 0·5 ml of 0·05 M-sodium phosphate buffer pH 7·4, containing 0·15 M-sodium chloride and 0·2 g/l sodium azide (PBS). The tubes were mixed and left at room temperature for a further 5 min before transferring the contents to a 1 × 30 cm column of Sephadex G-50. The ^{125}I-labelled IGFs were eluted with PBS containing 0·1 mg/ml BSA at a flow of approximately 10 ml/h. Typically, 78–91% incorporation of ^{125}I was achieved giving specific activities between 190 and 225 μCi/μg protein. The iodinated material was stored frozen and used within 10 days of iodination. At least 95% of the label was precipitable with 10% (vol/vol) trichloroacetic acid (TCA) on the day of use.

Lymph collection and plasma clearance of ^{125}I-IGF

The experiments were performed on twenty-five 3·5- to 8-week-old British Saanen male goats of 7·5–14 kg live weight. The animals had been weaned at least 10 days preceding the experiment and maintained on a diet of concentrates with hay and water *ab libitum*. The animals were allowed free access to their food up to 30 min before anaesthesia. Anaesthesia was induced and maintained with

sodium pentobarbitone (25 mg/kg) injected intravenously via a catheter (1·2 mm o.d., 0·8 mm i.d.; polypropylene tubing; Dural Plastics, NSW, Australia) inserted into the jugular vein. After intubation, oxygen inhalation was maintained in a closed circuit rebreathing apparatus with CO_2 removed by soda lime. A subclavian efferent lymph duct draining the foreleg was cannulated with polyvinyl tubing (0·8 mm o.d. 0·5 mm i.d.). The lymph drained into tared polystyrene tubes containing 10 μl of 0.5 M-disodium ethylenediaminetetra-acetic acid (EDTA) as an anticoagulant. The collecting tube was changed every 10 min and stored on ice until analysis. Lymph flow rate (ml/min) was determined gravimetrically assuming a lymph density of 1·01 g/ml.

^{125}I-IGF-I (4–18·5 μCi in 0·5 ml) was injected into the jugular vein of thirteen kid goats and ^{125}I-IGF-II (6–16 μCi in 0·5 ml) into twelve kids. Any material remaining in the catheter was flushed into the animal with sterile saline. Samples (1 ml) of arterial blood were withdrawn at 0·5, 1, 2, 3, 4, 5, 10 min and thereafter at 10 min intervals, coinciding with the changing of the lymph collection tubes, via a catheter (1.2 mm o.d., 0·8 mm i.d., polyethylene tubing) inserted into the carotid artery. The samples were put into polystyrene tubes containing EDTA, and plasma prepared by centrifugation (1500 *g*, 15 min). The sampling was continued for 4 h.

Total radioactivity present in 0·5 ml aliquots of plasma and weighed lymph samples was measured in a γ-counting system (Cobra 5005, Packard). Precipitation of undegraded peptide was achieved with 10% (final vol/vol) TCA.

Estimation of plasma volume

Plasma volume was estimated by dilution of 1·5–2 g of 1% (wt/vol) solution of Evans Blue dye injected intravenously (Little & Williams, 1964). Samples of arterial blood (2 ml) were taken at 3, 5 and 7 min following injection.

Gel filtration of plasma and lymph

Samples of plasma and lymph, taken after the radioactivity had reached maximum in lymph (60–80 min), were applied to a 1·8 × 35 cm column of TSK Fractogel HW-55(S) previously equilibrated with PBS (pH 7·4) containing 0·1 mg/ml BSA. Fractions of 0·5 ml were collected at a flow of 1 ml/min. The column was calibrated with Blue Dextran, ovalbumin, ^{125}I-IGF-I and potassium dichromate. Some samples of plasma and lymph were also chromatographed on a 0.5 × 30 cm column of Sephacryl S-200, equilibrated with PBS containing 0·1 mg/ml BSA. Fractions of 1 ml were collected at a flow of 0·5 ml/min. To determine whether the radioactivity eluting > 7000 Da was authentic IGF that was associated with binding proteins, plasma was diluted with 1 vol of 2 M-acetic acid (pH 2·8), mixed and left for 0·5 h at 4 °C. The sample was transferred onto a Sephadex G-75 column (0·5 × 30 cm) and eluted with 1 M-acetic acid (pH 2·5) at 0·5 ml/min. The elution position of authentic IGF was determined by chromatography of ^{125}I-IGF-I under the same conditions.

Ligand blotting

A modification of the Western blotting technique was employed to detect IGF binding activity in plasma and lymph. Samples of plasma (4 μl) and lymph (8 μl), supplemented with glycerol, Tris (pH 8·6), sodium dodecyl sulphate (SDS) and Bromophenol Blue, were electrophoresed under non-reducing conditions on 12% (wt/vol) polyacrylamide gel containing 0·1% SDS (wt/vol). The separated proteins were transferred electrophoretically to nitrocellulose membrane, which was treated exactly as described by Hossenlopp, Seurin, Segovia-Quinson, Hardouin & Binoux (1986) using ^{125}I-IGF-I or ^{125}I-IGF-II as the ligand.

Analysis of data

The clearance of TCA-precipitable radioactivity from plasma was fitted by non-linear regression analysis employing differential equations (Bevington, 1969). The half-lives and volumes of distribution of the two components of the decay curve were calculated according to the methods described by Tait, Tait, Little & Laumas (1960). In order to remove any variation arising from the use of animals with different body weights and therefore different volumes of body water, the radioactivity in lymph was expressed as a percentage of the calculated amount in plasma at time zero. This latter value was obtained by extrapolation of the decay curves to zero on the time axis using the equations generated from the individual animal's decay curve. Although the shapes of the subsequent

curves were not different from those generated from the raw data, this transformation allows more accurate assessment of the relative amounts of ^{125}I-labelled IGF-I or IGF-II transferred into lymph.

RESULTS

Plasma

Both ^{125}I-labelled IGF-I and IGF-II appeared in arterial plasma as TCA-precipitable and TCA-soluble radioactivity following their injection into the jugular vein. The time course of clearance of TCA-precipitable radioactivity from arterial plasma is shown in Fig. 1. The radioactivity present in plasma eluted from Fractogel HW-55(S) column, run at neutral pH, at positions corresponding to high molecular weight binding proteins, free IGF and the salt peak (Fig. 2). This indicates the presence of undegraded and degraded fragments of IGF. When plasma was subjected to gel filtration on Sephadex G-75 at acid pH, thereby disrupting the IGF–binding protein interaction, the major peak of radioactivity eluted as free ^{125}I-IGF. When corrected for procedural losses the amount eluting at this position was indistinguishable from the quantity of TCA-precipitable material in that sample, indicating this material was authentic IGF.

The proportion of total radioactivity in plasma which was TCA precipitable decreased progressively with time (Fig. 3). While there was no difference in this value for IGF-I and

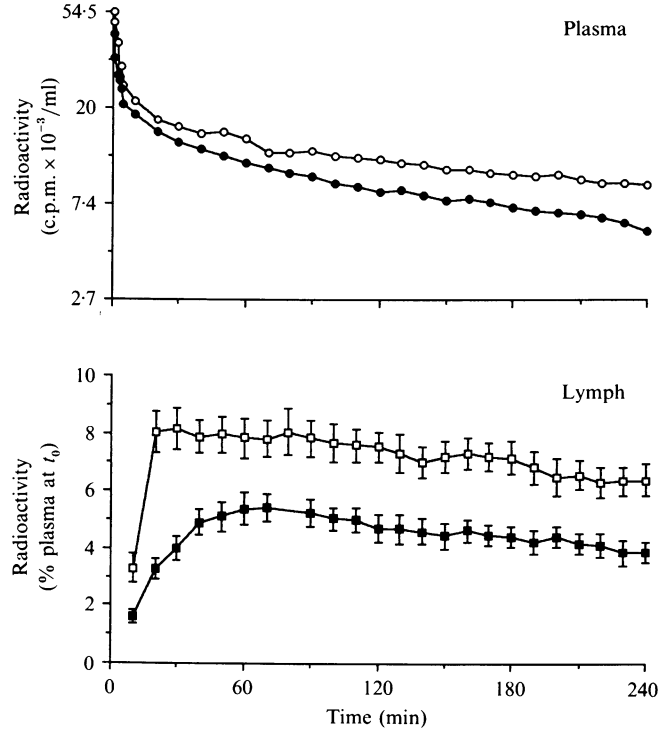

Fig. 1. Time course of clearance of trichloroacetic acid (TCA)-precipitable radioactivity from arterial plasma and appearance in lymph following intravenous injection of ^{125}I-labelled IGF-I (○, □) and IGF-II (●, ■) in 3·5- to 8-week-old goats. Levels of TCA-precipitable radioactivity in lymph of kid goats were expressed as a percentage of the amount in plasma at time zero (t_0) to reduce inter-animal variation. All values are means ± S.E.M. of thirteen experiments for IGF-I and twelve experiments for IGF-II. Note the use of a logarithmic scale for plasma radioactivity. Error bars for plasma data are smaller than the symbols and not shown.

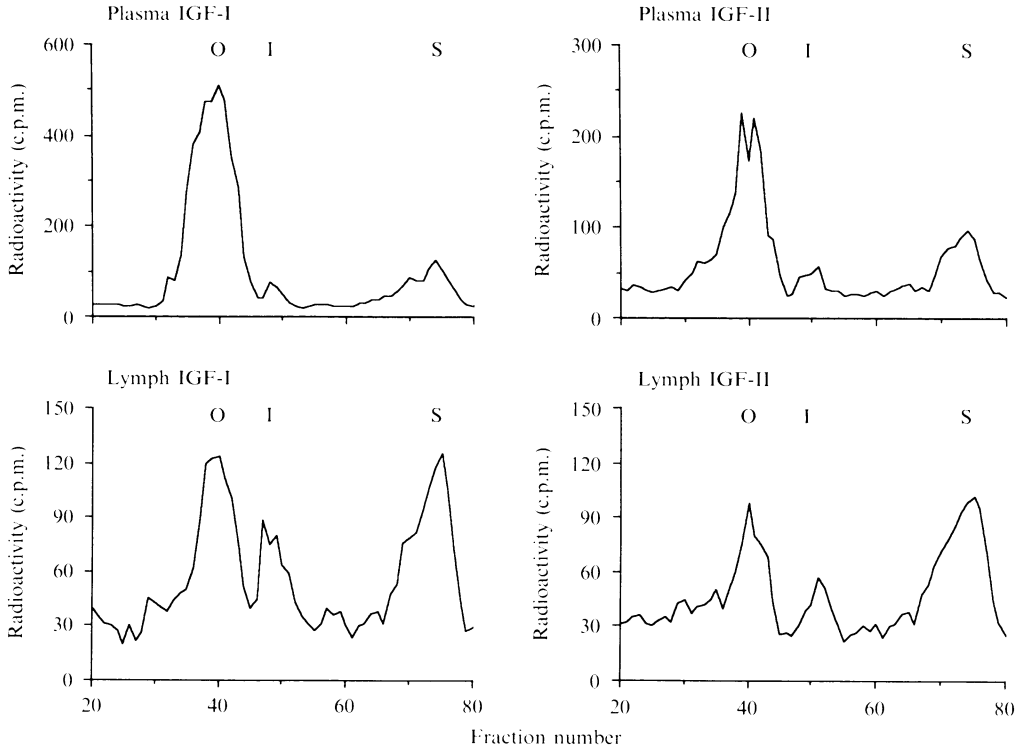

Fig. 2. Gel filtration of plasma collected at 60 min and lymph collected between 60 and 80 min after intravenous injection of ^{125}I-labelled IGF-I or IGF-II into kid goats. Samples were applied to a 1·8 × 35 cm TSK Fractogel HW-55(S) column and eluted in 0·5 ml fractions with sodium phosphate buffer (pH 7·4) containing 0·15 M-sodium chloride and 0·1 mg/ml bovine serum albumin. The elution positions of ovalbumin (O), ^{125}I-IGF-I (I) and potassium dichromate (S) which indicate bound and free IGFs and the salt peak, respectively, are shown.

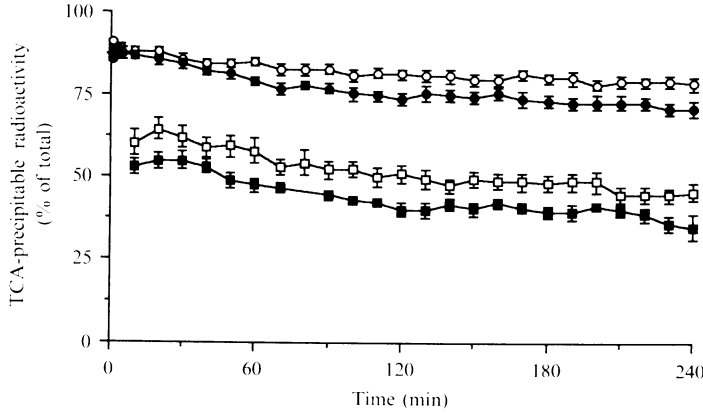

Fig. 3. Percentage of total radioactivity present in plasma (○, ●) or lymph (□, ■) which represented undegraded ^{125}I-IGF-I (○, □) or ^{125}I-IGF-II (●, ■). Undegraded IGF-I or IGF-II was measured by precipitability with 10% (final vol/vol) trichloroacetic acid (TCA).

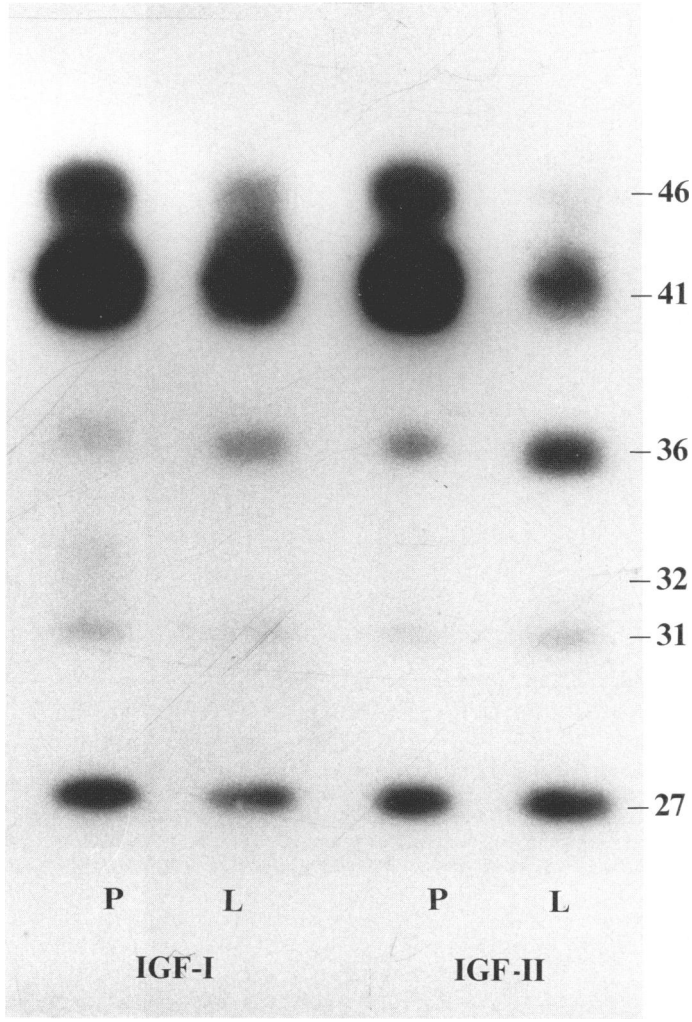

Fig. 4. Autoradiograph showing binding of ^{125}I-IGF-I and ^{125}I-IGF-II to proteins from lymph (L, 8 μl) or plasma (P, 4 μl) after separation on 12% polyacrylamide gel and electrotransfer to nitrocellulose. The numbers on the right are the molecular weights (in kDa) of the labelled proteins as calculated from positions of reduced standards run on the same gel.

IGF-II over the first 10 min, between 10 and 60 min the values for IGF-II declined more rapidly than IGF-I. By 60 min the differences between IGF-I and IGF-II were statistically significant ($P < 0{\cdot}05$, t test) and this was maintained for the remainder of the experiment.

The clearance of TCA-precipitable radioactivity, which represents authentic IGF, from plasma was best described by an exponential decay curve with two phases. The half-lives of these phases and the initial and final distribution volume of ^{125}I-labelled IGF-I and IGF-II are given in Table 1. The initial (0–10 min) volume of distribution for IGF-I and IGF-II were the same and agreed with the plasma volume of the goats as measured by dilution of Evans Blue. The half-lives of both components of the decay curve for IGF-II were significantly shorter than those of IGF-I. In contrast, the final distribution volume for IGF-II was 37% greater than that of IGF-I.

Table 1. *Half-lives and distribution volumes of ^{125}I-labelled IGF-I and IGF-II in 3·5- to 8-week-old goats*

	IGF-I	IGF-II
Half-life of component 1 (min)	9·6±0·9	4·2±0·6**
Half-life of component 2 (min)	287±23	172±16**
Initial distribution volume (ml/kg live weight)	45±3	52±5
Final distribution volume (ml/kg live weight)	90±10	125±12*
Plasma volume (ml/kg live weight)	42±2	50±3

The half-lives of the two phases for plasma clearance of peptides were calculated from the double exponential decay curves fitted to the data by regression analysis. Values are means±S.E.M. of thirteen experiments for IGF-I and twelve for IGF-II. Plasma volume, measured by dilution of Evans Blue, was measured in four animals from the IGF-I group, but all twelve animals from the IGF-II group. Differences between IGF-I and IGF-II were tested using unpaired *t* test; $*P < 0·05$, $**P < 0·001$.

To determine the association of undegraded ^{125}I-labelled IGF with plasma binding proteins, plasma taken between 60 and 80 min was subjected to gel filtration on Fractogel HW-55(S) at neutral pH. In confirmation of the report by Hodgkinson, Davis, Moore, Henderson & Gluckman (1989*a*) there was no evidence of dissociation of IGF-I or IGF-II from plasma binding proteins during storage. The percentage bound ^{125}I-IGF-I in plasma from freshly drawn blood was 92% and after 6 h at 20 °C or 8 h at 4 °C the percentages were 93 and 91% respectively. The corresponding values for ^{125}I-IGF-II were 94, 94 and 92% respectively. It was estimated that 91·1±0·9% of ^{125}I-IGF-I was associated with binding proteins, while 7±2% remained in the free form. The proportions of bound and free ^{125}I-IGF-II were 92±2 and 6±2% respectively.

Lymph

Like plasma, radioactivity in lymph consisted of TCA-precipitable and TCA-soluble material which eluted from Fractogel HW-55(S) at positions corresponding to bound and free IGF and at, or near, the salt peak for the column (Fig. 2). The amount of radioactivity eluting as bound and free IGF in lymph was identical to that which was TCA precipitable. The proportion of total radioactivity in lymph which was undegraded was much less than in plasma (Fig. 3). Like plasma, more of the radioactivity derived from IGF-II was present as degraded products than for IGF-I (Fig. 3).

The time course of appearance of TCA-precipitable radioactivity in lymph is given in Fig. 1. ^{125}I-IGF-I reached its peak in lymph by 20 min, but maximum values for ^{125}I-IGF-II were not attained until at least 60 min. After these times the amount of ^{125}I-IGF-I and ^{125}I-IGF-II declined very slowly at a similar rate for both peptides. At the times when maximum values for each peptide were obtained in lymph, the amount of ^{125}I-IGF-I was 50% greater than ^{125}I-IGF-II.

In lymph taken between 60 and 80 min after the injection of IGFs, 66±3% of ^{125}I-IGF-I was associated with binding proteins, while 34±2% was present in the free form. The corresponding values for ^{125}I-IGF-II were 77±3 and 23±3% respectively. The differences between IGF-I and IGF-II were significant at $P < 0·02$. The ratio of free ^{125}I-IGF-I in plasma to that in lymph was 1·4±0·3:1, whereas the same ratio for bound ^{125}I-IGF-I was 8·5±0·8:1. For ^{125}I-IGF-II these ratios were 1·5±0·3:1 and 8·1±1·3:1 respectively.

Ligand blotting

Ligand blotting of plasma and lymph revealed six binding species with approximate molecular weights of 46, 41, 36, 32, 31 and 27 kDa (Fig. 4). All IGF-binding proteins species present in plasma were found in lymph and detected with either IGF-I or IGF-II. However, the intensity of labelling was less in lymph, even though twice as much lymph (8 μl) was added compared with plasma (4 μl), indicating the latter contained substantially more IGF-binding activity. The pattern of labelling was also very similar for IGF-I or IGF-II, although the protein of molecular weight of 36 kDa exhibited a greater intensity of labelling using ^{125}I-IGF-II compared with ^{125}I-IGF-I.

DISCUSSION

The pattern of clearance of ^{125}I-IGF-I from plasma and its calculated half-life following its intravenous injection into 3- to 8-week-old goats is similar to that obtained in lambs (Francis, McNamara, Filsell & Ballard, 1988) and mature sheep (Hodgkinson, Davis, Burleigh, Henderson & Gluckman, 1987). The clearance of injected ^{125}I-IGF-II from kid goats and sheep (Hodgkinson *et al.* 1989*a*) follows essentially the same form, but with a significantly shorter half-life than IGF-I. In confirmation of the report of Francis *et al.* (1988) we found that clearance of ^{125}I-labelled IGF-I and IGF-II from plasma was associated with steadily increasing amounts of TCA-soluble material in the circulation. Column chromatography of plasma revealed this material probably represented degraded products of IGFs. These were presumably formed *in vivo* after internalization of the peptide by cells (Banskota, *et al.*, 1986; Bar, *et al.*, 1986; Schalch, Sessions, Farley, Masakawa, Elmer & Dills, 1986) and subsequent release of breakdown products back into the circulation or from degradation by ectoproteases present on the surface of endothelial cells. The greater proportion of TCA-soluble material in lymph compared to that in plasma is in keeping with the former hypothesis since the cellular breakdown products would be released firstly into the interstitial fluid which contributes to lymph composition.

The ^{125}I-IGF-I or IGF-II was rapidly (within 10 min) distributed into a volume identical to the plasma volume of the kid goats. The final volume of distribution, however, was far less than the extracelluar fluid space (approximately 20% of body mass), implying that these compounds have only restricted ability to cross the capillary endothelium. However, the presence of undegraded IGFs in lymph of the foreleg suggests transfer across the vascular endothelium of tissues in this region does occur *in vivo*.

Gel filtration revealed that between 60–80 min after their injection the IGFs were present in lymph as free and protein-bound peptide. Although the column resolution was not sufficient to allow direct comparison of the different binding proteins present in plasma or lymph, ligand blotting demonstrated the presence of identical proteins in lymph and plasma, albeit at much lower amounts in lymph. This binding profile for lymph from goats was also observed by Lord *et al.* (1991) for lambs and Davis, Hodgkinson, Prosser & Gluckman (1992) for sheep, but differs from human lymph (Binoux & Hossenlopp, 1988) in that the latter is virtually devoid of the doublet running at 41–46 kDa.

A significantly greater proportion of IGFs in lymph were present in the free form (34 ± 2% for IGF-I and 23 ± 2% for IGF-II) compared with plasma (7 ± 2 and 6 ± 2% for IGF-I and IGF-II respectively). Levels of free IGF-I and IGF-II present in plasma were less than twofold greater than those in lymph, similar to the plasma:lymph ratio for insulin in dogs (Yang, Hope, Alder & Bergman, 1989), but there was 8 times as much protein-bound

IGF-I and IGF-II in plasma as in lymph. The ability of binding proteins to maintain circulating levels of IGF-I has previously been demonstrated using analogues (Cascieri, Saperstein, Hayes, Green, Chicchi, Appelbaum & Bayne, 1988; Ballard, Knowles, Walton, Edson, Owens, Mohler & Ferraiolo, 1991), and reduced forms of this peptide (Francis *et al.* 1988) which have a much lower affinity for plasma binding proteins and are consequently cleared more rapidly. The present findings demonstrate that in the intact animal both IGF-I and IGF-II cleared from plasma is transferred into the extracellular fluid space of at least the foreleg in an undegraded form.

The basis for the retention of protein-bound IGF within the vascular space may relate simply to the greater exclusion of large molecular weight proteins from the normal routes of transendothelial transfer (Palade, Simionescu & Simionescu, 1979). Alternatively, if IGFs are transported by a receptor-mediated process similar to insulin (King & Johnson, 1985), the binding proteins might act to restrict binding of IGFs to insulin receptors. This would ensure that transfer is regulated by the relative presence within the capillary bed of specific receptors for IGF (Bar & Boes, 1984; Jialal, Crettaz, Hachiya, Kahn, Moses, Buzney & King, 1985). More recent evidence suggests that the binding proteins themselves might be instrumental in regulating transfer of IGFs across the capillary endothelium (Bar, Boes, Duke, Sandra, Bayne, Cascieri & Booth, 1990).

The present study highlights several major differences between the *in vivo* processing of plasma IGF-I and IGF-II. Firstly, IGF-II was distributed into a 37% larger volume at equilibrium compared with IGF-I. Secondly, it took 3 times longer for ^{125}I-IGF-II to reach maximum in lymph, although its clearance from plasma was more rapid than ^{125}I-IGF-I. Thirdly, 50% more ^{125}I-IGF-I transferred into lymph was TCA precipitable compared with ^{125}I-IGF-II, but significantly more of the latter was released into lymph and plasma in a degraded form.

The explanation of these differences may lie with different rates of uptake and degradation of the two growth factors. Since the composition of lymph is determined both by transfer of substances from the vascular space into interstitial fluid and cellular function (Palade, *et al.* 1979), if any substance is internalized by cells and only its degraded fragments released, the content of this substance in lymph will be reduced. As the proportion of TCA-soluble radioactivity in both lymph and plasma was greater for IGF-II than IGF-I we would predict that either more IGF-II was internalized by cells or it was more readily degraded. A greater cellular uptake of IGF-II may be inferred from its larger volume of distribution *in vivo* and studies *in vitro* which demonstrate more internalization and degradation of ^{125}I-IGF-II by capillary endothelial cells compared with ^{125}I-IGF-I (Bar *et al.* 1986). Thus, the more rapid clearance of ^{125}I-IGF-II from plasma as well as its slower equilibrium and lower concentration in lymph may be explained by its greater cellular uptake and/or degradation compared with ^{125}I-IGF-I. It is interesting to speculate that binding proteins present in plasma and lymph might play a central role in this process, given that these have differing affinities for the IGFs (Hodgkinson *et al.* 1989*b*) and can influence the availability of IGFs to cell receptors (see Ooi & Herington, 1988; Baxter & Martin, 1989*a*; Clemmons, 1991).

In conclusion, the present results provide direct experimental evidence for vectorial transfer of undegraded ^{125}I-labelled IGF-I and IGF-II from the circulation into the extracelluar fluid space of the foreleg of young goats. This is in keeping with the ability of these factors to act in an endocrine fashion in a group of tissues undergoing rapid growth. The finding that levels of IGF-I and IGF-II in lymph were maintained, declining only as those in plasma decreased, reinforces the proposed role of plasma binding proteins as

'storage' complexes, helping to maintain constant exposure of target tissues to IGF. Clearly, the mechanism of transfer of IGF across the vascular endothelium as well as its regulation requires further investigation.

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